

### Low-melt Polyester Embedding

This method was successful in our lab using prostate tissue and for our specific objectives. Investigators must be aware that they will need to tailor the following protocol for their own research objectives and tissue under study.

#### 1. Materials

1. 70%, 90%, 99%, 100% ethanol
2. Embedding molds, super metal based, 66 x 54 x15 mm (Surgapath Med. Ind. Inc.)
3. Embedding hardwood block, 1.5 x 1.5 x 1.0 inches (Shandon Lipshaw)
4. Microtome (Leica Microsystems Inc.)
5. Positively charged glass microscope slides (Brain Research Labs.)
6. Low-melt polyester (Gallard-Schlesinger Industries, Inc.)

#### 2. Methods

##### A: Large Tissue Specimens (several cm size)

1. After tissue fixation in 70% ethanol, place tissue in fresh 70% EtOH at 4°C for 2 hrs (x2).
2. Place tissue in 90% EtOH at 4°C for 1.5 hrs.
3. Place tissue in 99% EtOH at 4°C for 1.5 hrs.
4. Place tissue in absolute alcohol at 25°C for 2.5 hrs.
5. Melt the low-melt polyester at 38°C in an incubator and prepare two polyester:ethanol mixtures:
  - 50:50, polyester:100% ethanol
  - 90:10, polyester:100% ethanol

**TIP:** Do not use a microwave for heating nor exceed 45°C as these conditions will alter the properties of the polyester.

6. Infiltrate tissue in the above 50:50 polyester:ethanol mixture at 45°C for 2.5 hrs with agitation.
7. Infiltrate tissue in the above 90:10 polyester:ethanol mixture at 45°C overnight with agitation. Discard unused polyester after melting and do not reuse.

8. Transfer tissue to an embedding mold in 90:10 polyester:ethanol mixture and chill until a pellicle has formed on the surface of the polyester adjoining the tissue.
9. Trim the tissue block of excess polyester leaving a small margin around the tissue.
10. Attach the tissue block to a hardwood block by melting the back of the tissue block with a warm spatula and firmly pressing the two together.
11. Allow the tissue block to solidify at room temperature.
12. Store tissue blocks in the refrigerator at 4°C.

**B: Small Tissue Specimens and Biopsies (mm to 2 cm size)**

1. After tissue fixation in 70% ethanol, place tissue in fresh 70% EtOH at 4°C for 1 hr.
2. Place tissue in 90% EtOH at 4°C for 1 hr.
3. Place tissue in 99% EtOH at 4°C for 1 hr.
4. Place tissue in 100% alcohol at 25°C for 1 hr.
5. Infiltrate tissue in 50:50 polyester:ethanol mixture at 45°C for 2 hrs with agitation.
6. Infiltrate tissue in 90:10 polyester:ethanol mixture at 45°C for 1 hr with agitation.
7. Transfer tissue to plastic embedding mold in 90% polyester:absolute ethanol and chill on ice.
8. Store tissue blocks in the refrigerator at 4°C.

**C: Histology Slide Cutting Procedure**

1. Use a standard microtome to cut 8 µm thick sections.
2. Place sections in deionized water bath at room temperature.
3. Place the sections on positively charged glass slides and drain excess water.
4. Dry slides for 5 min at 30°C.
5. Store slides at 4°C until use.